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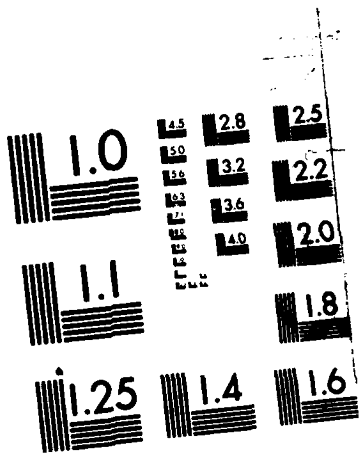


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Induced erythrocythemia is associated with an increase in maximal oxygen uptake. Two factors which might influence inter-subject variability for this increase in maximal oxygen uptake are the magnitude of change in hemoglobin concentration and the individual's initial aerobic fitness. To examine these factors, we have obtained and combined individual data from our own research and three other studies which employed similar procedures. In each study freeze-preserved erythrocytes representing the product of two blood units were reinfused and maximal oxygen uptake was measured within 24 to 72 h after reinfusion. The

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30 subjects had an initial aerobic power which ranged from 36 to 88 ml $O_2$ $kg^{-1}$ $min^{-1}$. The combined results from these studies indicate that after erythrocyte reinfusion: 1) the increase in hemoglobin concentration is fairly homogeneous; 2) nearly all individuals demonstrate an increase in maximal oxygen uptake; 3) the magnitude of increase in hemoglobin concentration is not related to the magnitude of increase in maximal oxygen uptake; and 4) the magnitude of increase in maximal oxygen uptake is related to the individual's initial aerobic fitness. Individuals with an initial aerobic fitness between 50 to 65 ml $O_2$ $kg^{-1}$ $min^{-1}$ experience approximately twice the increase in maximal oxygen uptake after erythrocyte reinfusion compared to individuals with lesser or greater fitness.

SPECIAL COMMUNICATION

# INDUCED ERYTHROCYTHEMIA AND MAXIMAL AEROBIC POWER: AN EXAMINATION OF MODIFYING FACTORS

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## ABSTRACT

Induced erythrocythemia is associated with an increase in maximal oxygen uptake. Two factors which might influence inter-subject variability for this increase in maximal oxygen uptake are the magnitude of change in hemoglobin concentration and the individual's initial aerobic fitness. To examine these factors, we have obtained and combined individual data from our own research and three other studies which employed similar procedures. In each study freeze-preserved erythrocytes representing the product of two blood units were reinfused and maximal oxygen uptake was measured within 24 to 72-h after reinfusion. The 30 subjects had an initial aerobic power which ranged from 36 to 88 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$. The combined results from these studies indicate that after erythrocyte reinfusion: 1) the increase in hemoglobin concentration is fairly homogeneous; 2) nearly all individuals demonstrate an increase in maximal oxygen uptake; 3) the magnitude of increase in hemoglobin concentration is not related to the magnitude of increase in maximal oxygen uptake; and 4) the magnitude of increase in maximal oxygen uptake is related to the individual's initial aerobic fitness. Individuals with an initial aerobic fitness between 50 to 65 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ experience approximately twice the increase in maximal oxygen uptake after erythrocyte reinfusion compared to individuals with lesser or greater fitness. Key words;

Index Terms: blood "doping", blood reinfusion, ergogenic aids, exercise performance, hemoglobin concentration, maximal oxygen uptake.

Several Olympic competitors have been thought to use blood infusions (homologous and autologous) or "blood doping" as an ergogenic aid. Recently, induced erythrocythemia was reported to increase an individual's maximal aerobic power (1,5,6,7,8,10) and submaximal exercise performance (1,12). To induce erythrocythemia, investigators in these studies reinfused the subjects with autologous freeze-preserved erythrocytes which represented the product of two blood units. The physiological mechanism believed primarily responsible for the increased maximal aerobic power is an elevated arterial oxygen content (3); however, other mechanisms such as blood volume expansion (3,5), increased maximal cardiac output (10) and improved blood buffering capacity (1) have been suggested to contribute to the ergogenic effect. Regardless of the physiological mechanism(s) responsible, surprisingly little detailed information is available which describes the magnitude of increase in maximal aerobic power elicited by induced erythrocythemia.

Several important questions concerning the ergogenic effects of induced erythrocythemia have not been addressed. For example, it is not known if the post-infusion increase in maximal aerobic power is: 1) evident in all individuals; 2) related to the change in hemoglobin concentration; and 3) modified by the individual's initial level of aerobic fitness. Answers to these questions might be obtained from an examination of existing data; however, integration of these data are difficult because only mean data of the pertinent variables are presented in most reports (1,5,6,7). However, through personal communication with several laboratories (1,6), we have obtained and combined individual data from three studies (1,6,10) as well as our own research (8) on the effects of blood-reinfusion on maximal aerobic power.

This paper examines inter-subject variability for the erythrocyte induced increase in maximal oxygen uptake, and addresses the extent to which this increase is related to changes in the individual's hemoglobin concentration, as well as modified by the individual's initial aerobic fitness.

## METHODS

Data were compiled from four investigations to examine the influence of erythrocyte reinfusion on maximal aerobic power (1,6,8,10). These investigations all employed similar procedures in which: 1) the reinfused autologous erythrocytes were the product of two blood units; 2) the erythrocytes were freeze preserved (11); 3) the reinfusion did not precede re-establishment of normocythemia; and 4) the maximal oxygen uptake was measured within 24 to 72-h after reinfusion. This time period enabled sufficient equilibration of body fluids between compartments after the reinfusion, and is well within the period of peak ergogenic effects (3).

Subjects. Table 1 presents a description of the 30 subjects who participated in the four investigations and whose data have been used in the present statistical analyses. The male subjects from Study I (1) were national and international caliber track athletes who were very-fit. Studies II (.0) and III (8) used moderately-fit male populations. We were unable to find published data on the influence of erythrocyte reinfusion on relatively unfit male populations. Data were available, however, on the effects of erythrocyte reinfusion for a female population with low-fitness (6). Despite the existence of gender differences for maximal aerobic power and hemoglobin concentration, we decided to include this female population (Study IV) in our statistical analyses. This enabled us to examine a population emcompasing very-fit (Study I), moderately-fit (Study II and III) and low-fit (Study IV) individuals.

Protocol. Study I (1) employed a double-blind design to determine the effects of acute erythrocythemia on maximal aerobic power. Two units of blood were removed by phlebotomy and stored by the glycerol freezing technique (11). After the reestablishment of normocythemia, the subjects were tested for their maximal aerobic power, reinfused, and tested again 24-h post-reinfusion. The

subjects were reinfused with the erythrocyte product of two blood units suspended in a saline solution to produce a hematocrit of approximately 50%. A supramaximal treadmill protocol was used to determine maximal oxygen uptake. Subjects ran to exhaustion at a treadmill level and speed that was calculated to elicit 110% of their pre-reinfusion maximal oxygen uptake.

During Study II (10) the subjects had two units of blood removed by phlebotomy and stored by the glycerol freezing technique. Approximately 3 months later, the subjects were reinfused with the erythrocyte product of these two blood units. Maximal oxygen uptake was measured 4-7 days pre- and 72-h post-reinfusion during a modified Balke treadmill test. The initial treadmill grade was zero and the grade was increased by 5% for the initial two 3-min exercise bouts and by 2% for each subsequent 3-min exercise bouts. During each exercise bout, the subjects walked at 3.5 $m \cdot s^{-1}$ and the criteria of Talyor et al. (9) were used to establish maximal oxygen uptake.

Study III (8) employed a double-blind design with a blood reinfusion and control group. Two units of blood were removed by phlebotomy and stored by the glycerol freezing technique. After the reestablishment of normocythemia, the subjects were tested for their maximal oxygen uptake and were tested again 72-h post-reinfusion. The subjects were reinfused with the erythrocyte product of two blood units suspended in a sodium chloride-glucose-phosphate solution to produce a hematrocrit of ~50%. Maximal oxygen uptake was determined from a progressive intensity, continuous effort treadmill test. The warm-up bout consisted of four minutes of walking (1.56 $m \cdot s^{-1}$) at a 4% treadmill grade. The subjects then ran (3.13 $m \cdot s^{-1}$) continuously at an initial grade of 5% with 2-1/2 % increments every two minutes, and the criteria of Talyor et al. (9) were used to establish maximal oxygen uptake.

During Study IV the subjects had two units of blood removed by phlebotomy and stored by the glycerol freezing technique. After the reestablishment of normocythemia the subjects were tested for their maximal oxygen uptake, reinfused, and were then tested again 48-h post-reinfusion. The subjects were reinfused with the erythrocyte product of two blood units suspended in a saline solution to produce a hematocrit of ~70%. Maximal oxygen uptake was determined during a continuous cycle ergometer test (50 rev$\cdot$min$^{-1}$). The initial power output was 73 W with increments of ~25 W after each 3-minute exercise bout.

Statistical Analyses. Simple linear regression and analysis of variance procedures were employed. Critical differences were calculated by Tukey's procedure. Statistical significance was tested at the $P < 0.05$ level. All presented values represent means and their standard deviations.

## RESULTS

Figure 1 presents the individual data for maximal aerobic power (maximal oxygen uptake in ml$\cdot$kg$^{-1}\cdot$min$^{-1}$) measured before and shortly after (24-to-72-h) erythrocyte reinfusion. Maximal aerobic power was increased ($P < 0.01$) from $60.6 \pm 16.3$ to $65.4 \pm 16.1$ ml $O_2\cdot$kg$^{-1}\cdot$min$^{-1}$, and Groups II and III had a greater ($P < 0.05$) increase than Groups I and IV from pre- to post-reinfusion. A correlation coefficient of $r=0.99$ ($P < 0.01$) was found between the pre- and post-reinfusion values. An increased maximal aerobic power was observed in 29 of 30 subjects, while a 43 year old individual failed to show an increment. To our knowledge, this person is the oldest to have received an erythrocyte reinfusion and subsequently to have his maximal oxygen uptake measured. The 43 year old individual's data have not been included in the statistical analyses of Figures 2 and 3, but is plotted for reference.

Figure 2 presents the relationship between an individual's initial aerobic power (maximal oxygen uptake in $ml \cdot kg^{-1} \cdot min^{-1}$) and the percent increase for that value subsequent to erythrocyte reinfusion. An analysis of variance indicated a difference ($P < 0.01$) among the groups for the percent increase in maximal aerobic power. Group I had a smaller ($P < 0.05$) percent increase than Groups II, III and IV. This smaller improvement for "very-fit" individuals, however, may reflect a bias from a larger denominator in the percent increase calculation. To adjust for this possible bias, Figure 3 presents the relationship between an individual's initial aerobic power and the absolute ($l \cdot min^{-1}$) increase in maximal oxygen uptake subsequent to erythrocyte reinfusion. An analysis of variance indicated a difference ($P < 0.01$) among the groups for the absolute increment in maximal oxygen uptake. Group I had a smaller ($P < 0.05$) increase than Group III, and Group IV had a smaller ($P < 0.05$) increase than Groups II and III. These data suggest that moderately-fit (Groups II and III) individuals have an accentuated increase in maximal oxygen uptake after erythrocyte reinfusion.

Hemoglobin data were available for Groups I, II, and III. Erythrocyte reinfusion resulted in an increased ($P < 0.01$) hemoglobin concentration ($1.36 \pm 0.55$g $Hb \cdot 100$ ml of $blood^{-1}$) which corresponds to a $10 \pm 5$ percent increase. No differences were found among the three studies for the increase in hemoglobin concentration after reinfusion. Figure 4 presents individual data for the percent change in hemoglobin and the percent change in maximal aerobic power values. Figure 5 presents the individual data for the absolute change in hemoglobin and the absolute change in maximal aerobic power ($\ell\ O_2 \cdot min^{-1}$) values. It can be noted that the 43 year old individual, who was the only individual that did not increase his maximal aerobic power, increased his hemoglobin concentration by only 0.7 g $Hb \cdot 100$ ml of blood $^{-1}$. Table 2 presents the correlation coefficients between the subjects' change in hemoglobin and change in maximal oxygen

uptake ($\ell$ $O_2 \cdot min^{-1}$) after erythrocyte reinfusion. No significant relationships were found between these variables.

## DISCUSSION

The factors which may modify the increase in maximal oxygen uptake induced by erythrocyte reinfusion have not been fully elucidated. This report has presented individual data from 30 subjects (1,6,8,10) who represent a range from low- to very- high aerobic fitness. Nearly identical methods were used for blood storage and reinfusion, but some differences existed among the protocols used to elicit maximal oxygen uptake. Therefore, some inter-study differences might exist for the maximal oxygen uptake increments. These four studies, however, were all conducted by experienced investigators in established laboratories and we have confidence in the comparability of these values. It should be noted, however, that the low-fit group were females and that collaborative data should be obtained from a group of low-fit males.

Erythrocyte reinfusion increased hemoglobin concentration by approximately 1.36 $g \cdot 100ml$ of $blood^{-1}$ which corresponds to an increased arterial oxygen content of ~1.82 ml $O_2 \cdot 100$ ml of $blood^{-1}$ (1.36 g Hb x 1.34 ml $O_2 \cdot g$ $Hb^{-1}$). During maximal exercise eliciting a theoretical cardiac output of 30 $\ell \cdot min^{-1}$, this would result in an additional 0.546 $\ell$ $O_2 \cdot min^{-1}$ being available to the tissues after erythrocyte reinfusion. Also, if erythrocyte reinfusion increased maximal cardiac output (3,10), then this theoretical volume of oxygen available to the tissues would be accentuated. However, an increased maximal oxygen uptake is also dependent upon the peripheral tissues ability to extract and utilize the additional oxygen which is made available (4). Overall, erythrocyte reinfusion increased maximal oxygen uptake by an average of $0.357 \pm 0.216$ $\ell \cdot min^{-1}$ which represents ~65% of the theoretical maximal potential for increase at a cardiac output of 30 $\ell \cdot min^{-1}$.

Surprisingly, there were no significant relationships between the increase in hemoglobin and the increase in maximal oxygen uptake (Table 2). The lack of significant relationships may, in part, be due to the homogeneity in the volume of infused erythrocytes; each subject received the product of two blood units. However, this is unlikely since the range of increase for hemoglobin concentration after erythrocyte reinfusion was fairly wide (from 0.6 to 2.6 g Hb·100 ml of blood$^{-1}$). Alternatively, individual differences may exist for the amount of additional oxygen which is made available to the contracting skeletal musculature during maximal exercise. For example, erythrocyte reinfusion may cause varied effects on maximal cardiac output (6,10) as well as vasomotor responses (at a given cardiac output) directing blood to the contracting musculature. Furthermore, some of the subjects may not have sufficient peripheral tissue adaptations, such as available capillary to fiber exchange surface area or enzymatic oxidative potential, to use the additional oxygen which is available (2,4).

Erythrocyte reinfusion increased maximal oxygen uptake for 29 of the 30 subjects. The magnitude of the increase in maximal oxygen uptake was related to the subject's initial fitness level. Individuals with an initial aerobic power between ~50 to ~65 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ appear to have the greatest response to erythrocyte infusion (Figure 4). The nine subjects in Groups II and III who responded to erythrocyte infusion increased their maximal oxygen uptake by $0.515 \pm 0.204$ ℓ·min$^{-1}$ which represents about ~94% of the theoretical maximal potential for increase. These moderately-fit individuals were probably sufficiently trained to have both the central (2) and peripheral (4) reserves to optimally use the increased arterial oxygen content after erythrocyte reinfusion.

Individuals with an initial aerobic power below 50 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ and above 65 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ displayed a very homogeneous but blunted increase in

maximal oxygen uptake after erythrocyte reinfusion (Figure 4). Both the low-fit (Group IV) and extremely-fit (Group I) individuals demonstrated approximately 50% of the increased maximal oxygen uptake shown by the moderately-fit individuals. Group IV and Group I increased their maximal oxygen uptake by $0.261 \pm 0.080$ and $0.249 \pm 0.046$ $\ell \cdot min^{-1}$, respectively. The physiological mechanism(s) responsible for the blunted increase in maximal oxygen uptake after erythrocyte reinfusion are probably different for each group. The low-fit individuals may not have the central reserves (ability to increase and redistribute the cardiac output) and peripheral reserves (ability to extract and utilize oxygen at the skeletal muscle) to fully use the increased arterial oxygen content during maximal effort exercise. In contrast, the very-fit individuals might already be effectively using (without erythrocyte reinfusion) nearly all of their potential central and peripheral reserves during maximal exercise.

In conclusion, we have analyzed individual data from four research studies on the influence of erythrocyte reinfusion on maximal oxygen uptake. The combined results from these studies indicate that for individuals reinfused with a volume of erythrocytes representing the product of two blood units that: 1) the increase in hemoglobin concentration is fairly homogeneous; 2) nearly all individuals demonstrate an increase in maximal oxygen uptake; 3) the magnitude of increase in hemoglobin concentration is not related to the magnitude of increase in maximal oxygen uptake; and 4) the magnitude of increase in maximal oxygen uptake is related to the individual's initial aerobic fitness. Individuals with an initial aerobic fitness between 50 to 65 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ experience approximately twice the increase in maximal oxygen uptake after erythrocyte reinfusion compared to individuals with lesser or greater fitness.

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The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation. Approved for public release; distribution unlimited.

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TABLE 1. Description of the subject population and test methods.

| Study | | n | Gender | Age (yr) | Height (cm) | Weight (kg) | Percent Body fat | Exercise Mode | Initial Maximal Aerobic Power ($ml \cdot kg^{-1} \cdot min^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| I. Buick et al. (1) | | | | | | | | | |
| | $\bar{X}$ | 11 | M | 21 | 175 | 64 | 7 | TM | 80 |
| | SD | | | 3 | 4 | 5 | 1 | | 6 |
| II. Thomson et al. (10) | | | | | | | | | |
| | $\bar{X}$ | 4 | M | 23 | 177 | 71 | - | TM | 56 |
| | SD | | | 1 | 7 | 6 | - | | 7 |
| III. Sawka et al. (8) | | | | | | | | | |
| | $\bar{X}$ | 6 | M | 30 | 182 | 79 | 15 | TM | 54 |
| | SD | | | 7 | 4 | 9 | 5 | | 5 |
| IV. Robertson et al. (6) | | | | | | | | | |
| | $\bar{X}$ | 9 | F | 23 | 167 | 56 | - | CY | 43 |
| | SD | | | 2 | 7 | 5 | - | | 4 |

TM is treadmill exercise and CY is cycle exercise

TABLE 2. Correlation coefficients between the subjects' change in hemoglobin (Hb) and change in maximal oxygen uptake ($\dot{V}O_{2max}$) after erythrocyte reinfusion.

| | | % Δ Hb vs. % Δ $\dot{V}O_2$ max | Δ Hb vs. Δ $\dot{V}O_2$ max | Δ Hb vs. % Δ $\dot{V}O_2$ max | % Δ Hb vs. Δ $\dot{V}O_2$ max |
|---|---|---|---|---|---|
| STUDY I(1) (n=11) | r = | 0.06 | 0.07 | -0.05 | 0.07 |
| STUDY II(10) (n=4) | r = | -0.63 | -0.65 | -0.53 | -0.74 |
| STUDY III(8) (n=6) | r = | 0.50 | 0.48 | 0.25 | 0.54 |
| Total (n=21) | r = | 0.27 | 0.11 | 0.14 | 0.35 |

Δ Hb is change in hemoglobin ($g \cdot 100ml^{-1}$); % Δ Hb is percent change in hemoglobin; Δ $\dot{V}O_2$ is change in maximal oxygen uptake ($\ell \cdot min^{-1}$); % Δ $\dot{V}O_2$ is percent change in maximal oxygen uptake.

## FIGURE LEGENDS

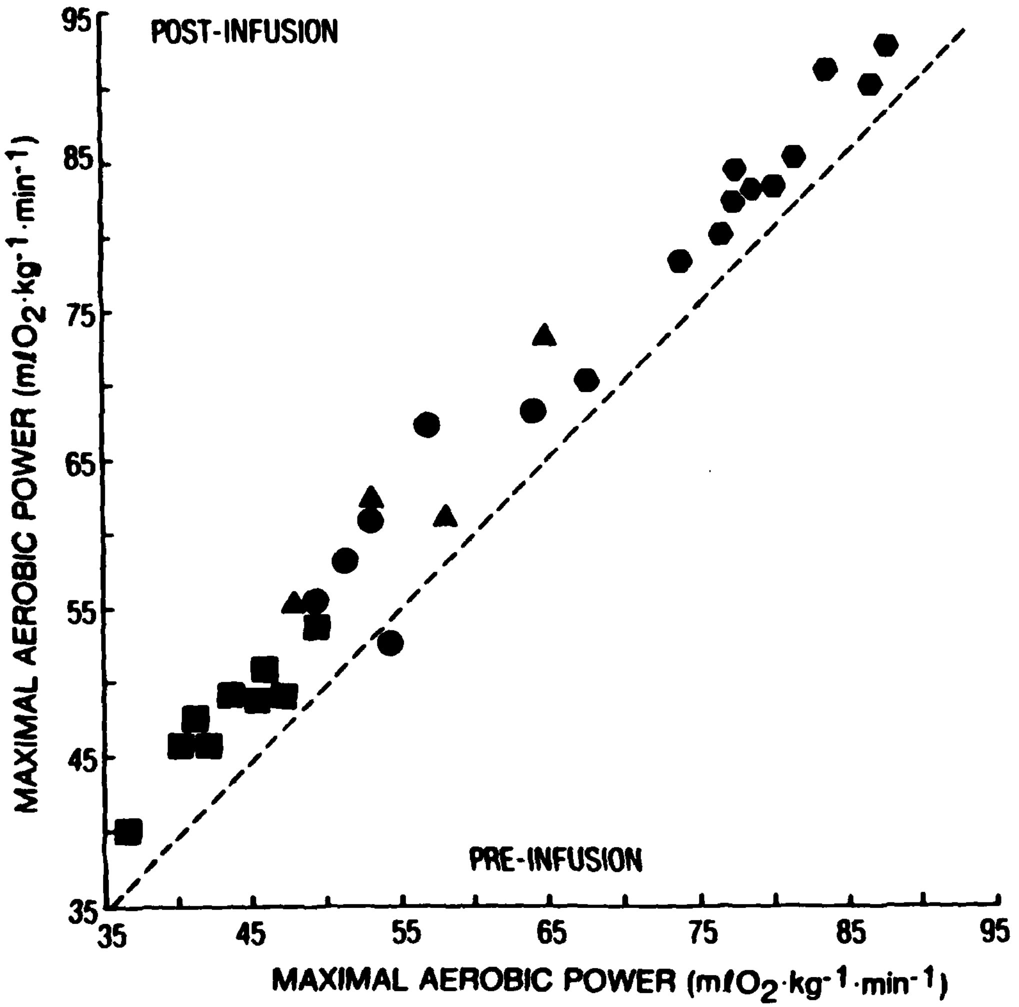
Fig. 1. Individual data for maximal aerobic power values measured before and after erythrocyte reinfusion. The broken line represents the line of equality. Group I (1) is ⬢, Group II (10) is ▲, Group III (8) is ● and Group IV (6) is ■. Note that the only individual failing to show an increased maximal aerobic power was 43 years old.

Fig. 2. Individual data for the relationship between initial (pre-reinfusion) maximal aerobic power and the percent increase in maximal oxygen uptake after erythrocyte reinfusion. Group captions are the same as for Fig. 1. The broken line represents no change in aerobic power.

Fig. 3. Individual data for the relationship between initial (pre-reinfusion) maximal aerobic power and the absolute ($\ell\ O_2\cdot min^{-1}$) increase in maximal aerobic power after erythrocyte reinfusion. Group captions are the same as for Fig. 1. The broken line represents no change in aerobic power.

Fig. 4. Individual data for the relationship between percent change in hemoglobin and percent change in maximal aerobic power after erythrocyte reinfusion. Group captions are the same as for Fig. 1. The broken line represents the line of equality.

Fig. 5. Individual data for the relationship between the absolute change in hemoglobin and absolute change in maximal aerobic power after erythrocyte reinfusion. Group captions are the same as for Fig. 1. The broken line represents no change in aerobic power.


POST-INFUSION
PRE-INFUSION
MAXIMAL AEROBIC POWER ($m\ell O_2 \cdot kg^{-1} \cdot min^{-1}$)
MAXIMAL AEROBIC POWER ($m\ell O_2 \cdot kg^{-1} \cdot min^{-1}$)
95
85
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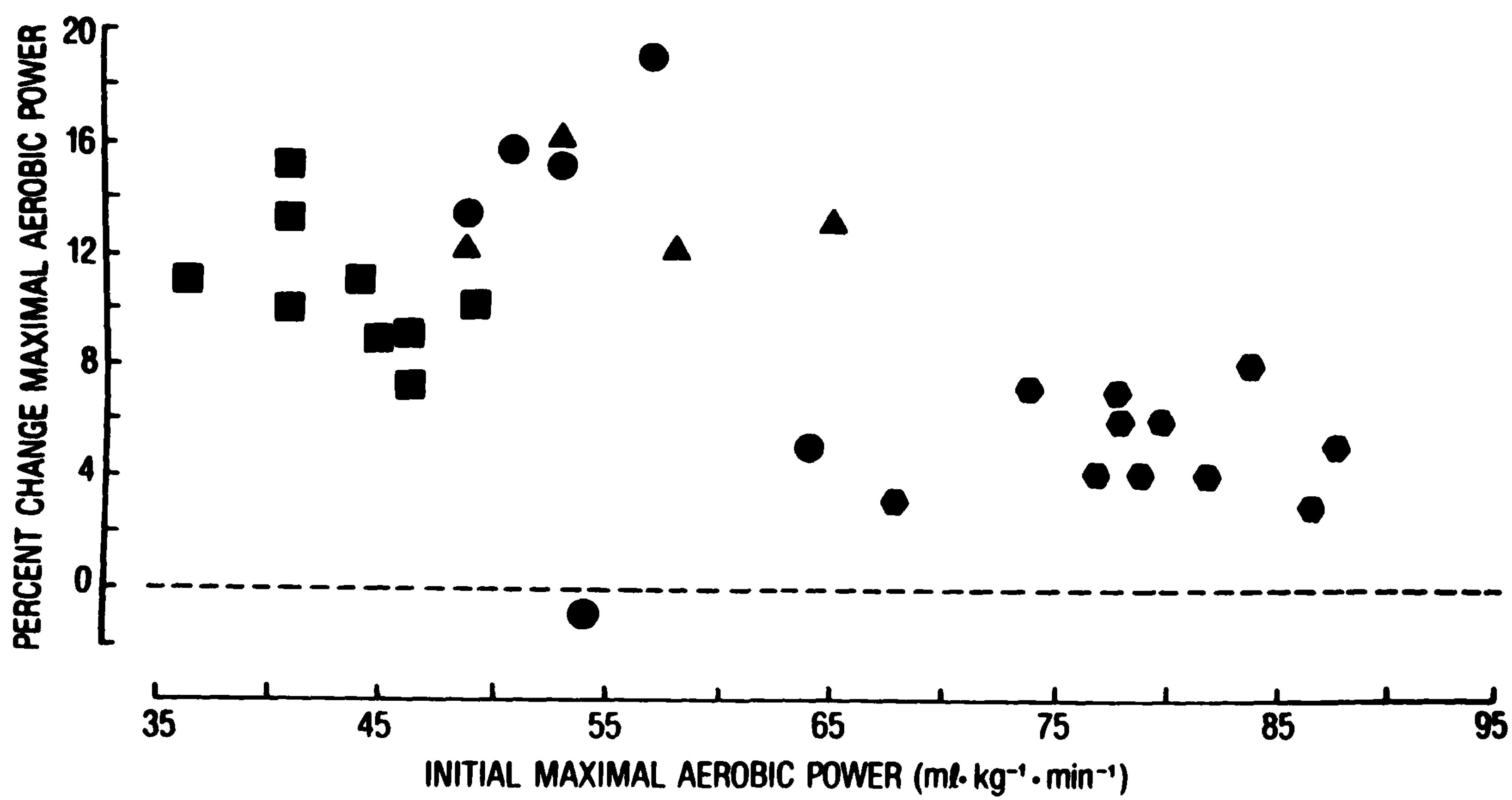
PERCENT CHANGE MAXIMAL AEROBIC POWER
20
16
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8
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45
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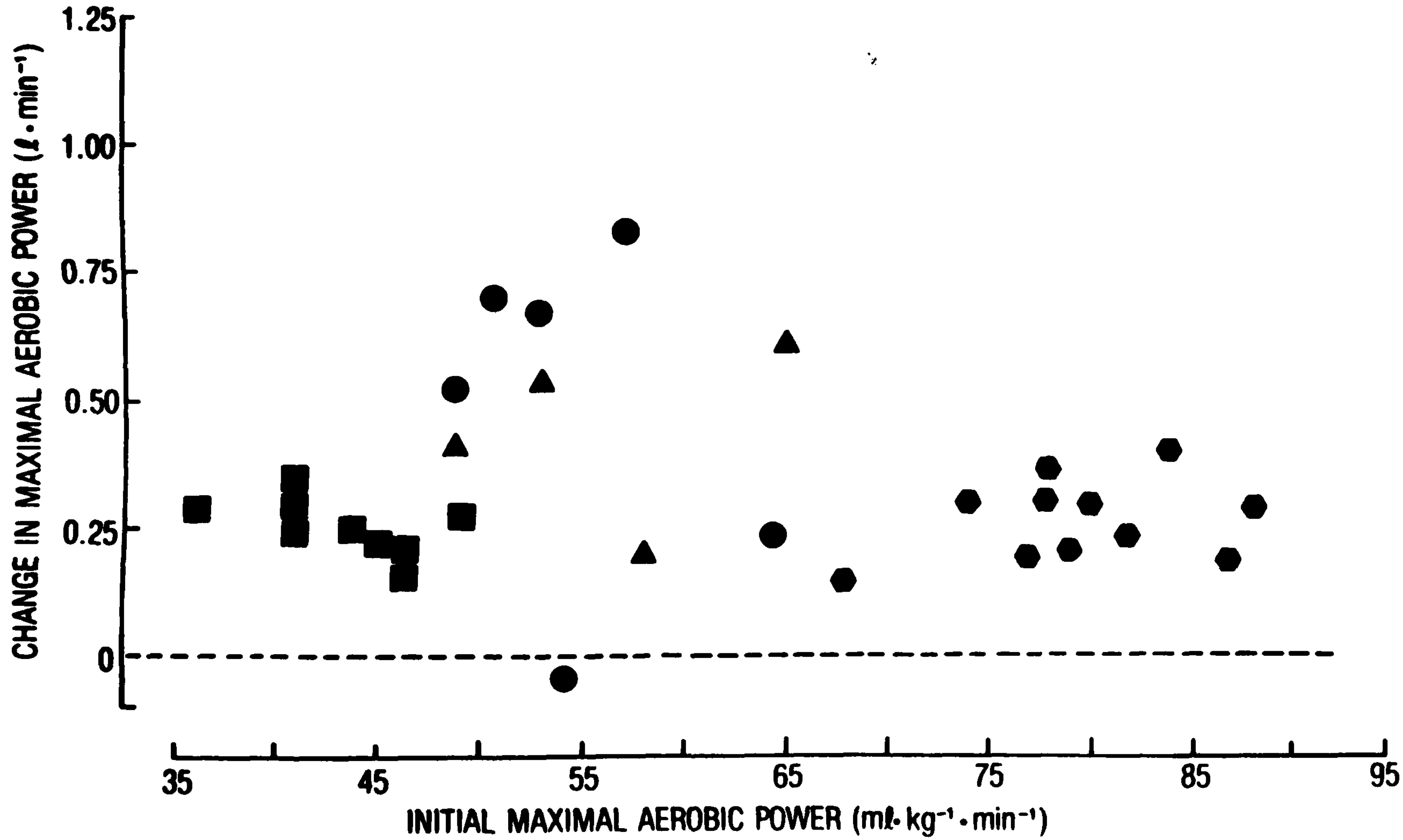
75
85
95
INITIAL MAXIMAL AEROBIC POWER (ml·kg⁻¹·min⁻¹)



CHANGE IN MAXIMAL AEROBIC POWER (ℓ · min⁻¹)
1.25
1.00
0.75
0.50
0.25
0
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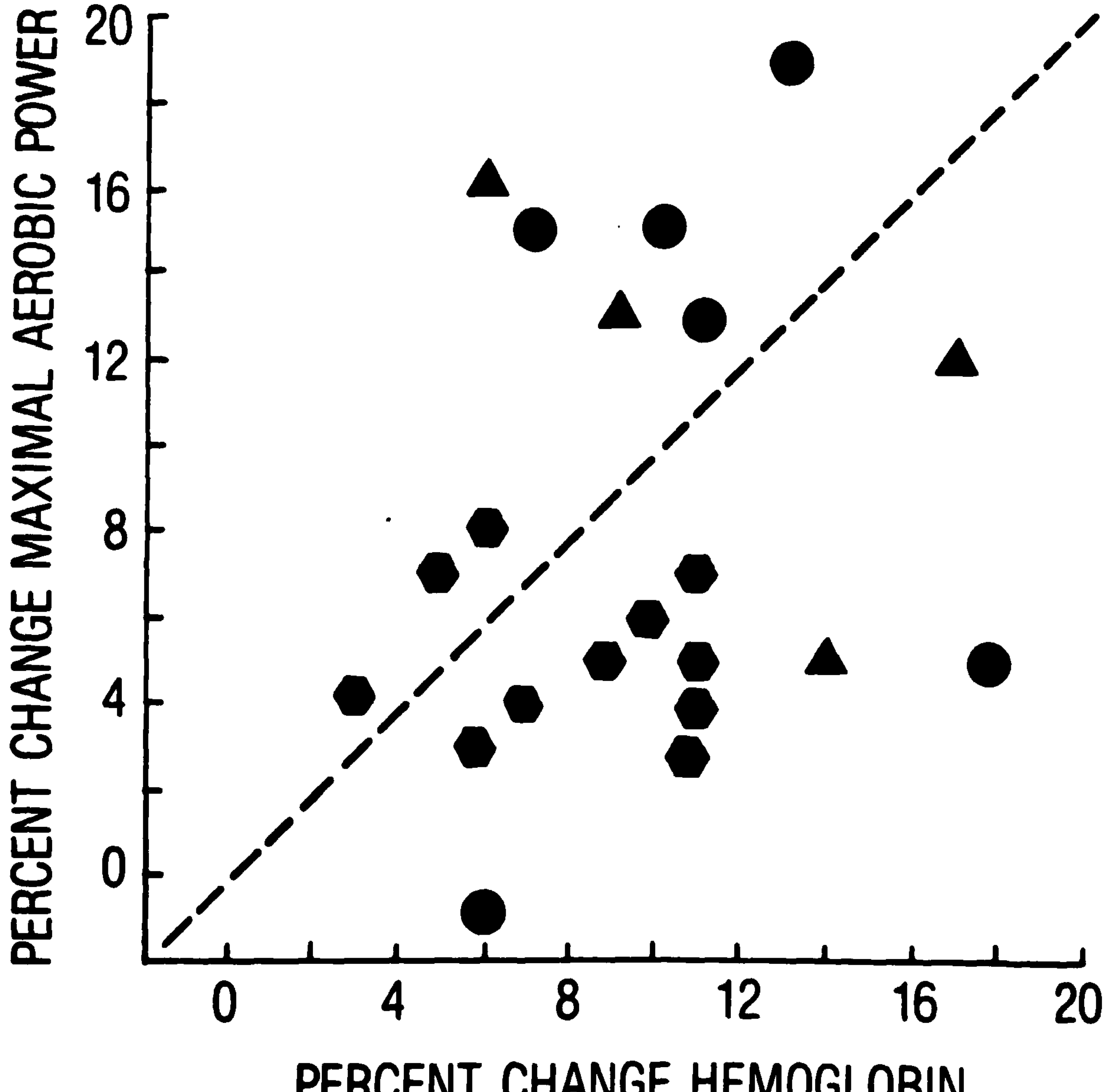
65
75
85
95
INITIAL MAXIMAL AEROBIC POWER (mℓ · kg⁻¹ · min⁻¹)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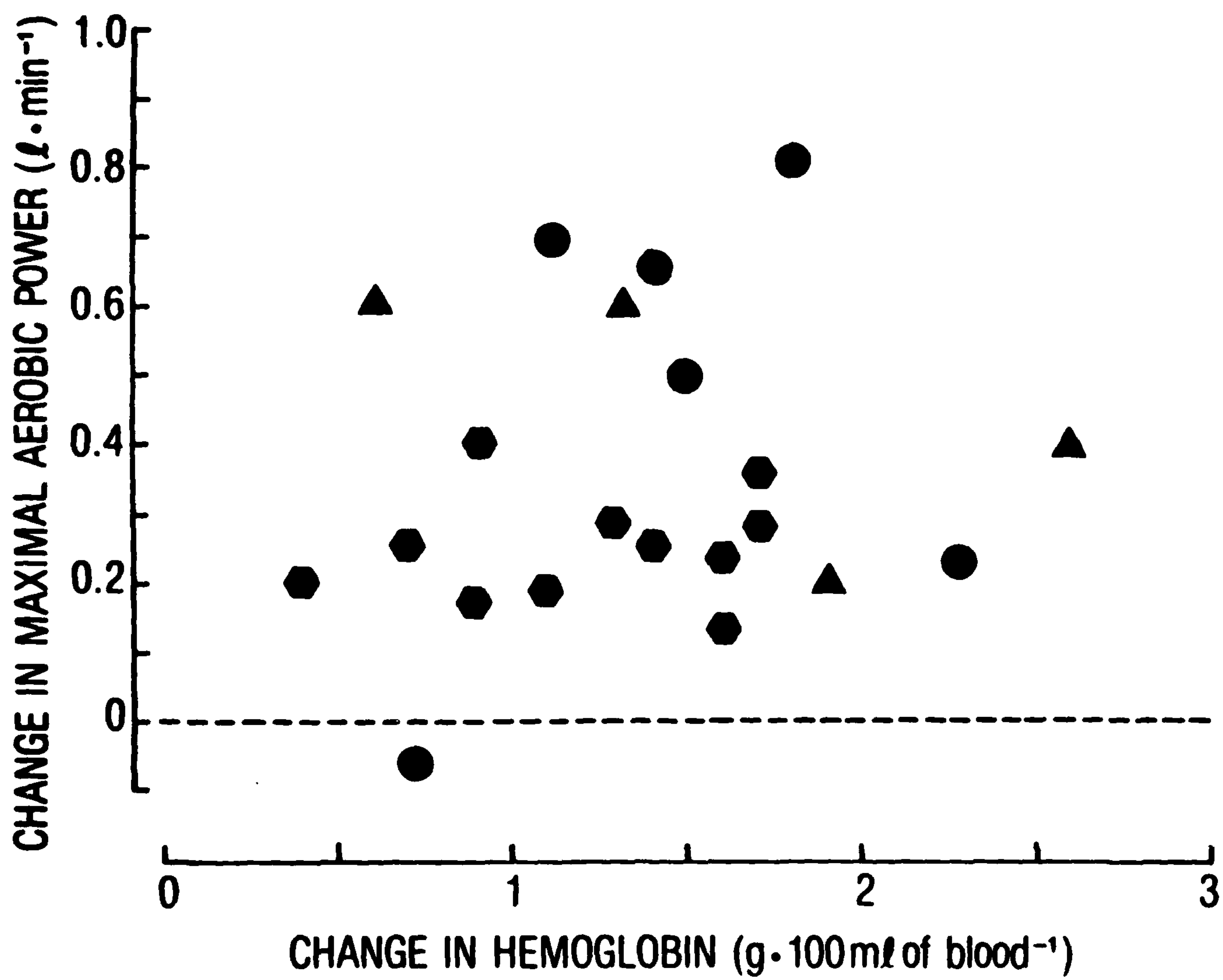
PERCENT CHANGE HEMOGLOBIN



CHANGE IN MAXIMAL AEROBIC POWER (ℓ·min⁻¹)
1.0
0.8
0.6
0.4
0.2
0
0
1
2
3
CHANGE IN HEMOGLOBIN (g·100mℓ of blood⁻¹)

END

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